Cocoa bean shell waste valorisation; extraction from lab to pilot-scale cavitational reactors

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Abstract

The use of zero-waste processes to integrate food-waste valorisation into the circular economy equation is currently one of the hottest topics in sustainability research. This goal is still far from being fully achieved despite the release of a number of patents and papers that deal with the topic.

The present work aims to valorise cocoa shells, one of the main by-product of the roasting process, in order to enhance the effective extraction of high added value compounds by means of green protocols. The high potential added value of the residual waste has been demonstrated via a direct analytical comparison of extracts and bean composition.

A range of raw matrix extraction procedures have been investigated in order to define the best solvent and technology; ultrasound (US) and hydrodynamic cavitation (HC) were compared with conventional methods. The high-energy microenvironments generated by cavitation substantially promote fast biomass deconstruction with low energy consumption. The optimized protocol couples

a HC reactor with a ternary water/ethanol/hexane mixture, simultaneously providing a hydrophilic product, which is rich in methylxanthines and polyphenols, and a lipid layer.

Sequential milling and sieving pretreatment provided an enriched shell fraction via the partial removal of husk fibres (54.45 *vs*. 81.36 w/w % total fibres). The disposal of the latter reduces mass balance, but is rerouted into animal feedstock components and crop mulching.

The protocols herein reported produce valuable extracts, which are rich in antioxidant flavanols (catechins and epicatechins), theobromine (32.7±0.12 mg/g shells), caffeine (1.76±0.08 mg/g shells) and cocoa butter, in a simple and easy manner. This new valorisation process afforded 20.5 w/w % and 15.8 w/w % hydrophilic and lipophilic fractions, respectively, when scaled up to function in a pilot flow reactor. The fatty acids, obtained in remarkable yield (forming the 96.4 w/w % of the total light part) well match the commercial cocoa butter profile. The antioxidant extract shows an impressive total phenolic content of 197.4 mg/g extract (gallic acid eq.), with a radical scavenging activity of 62.0±3.1 μg/mL (expressed in DPPH EC_{50}). This work should facilitate industrial design for the convenient recovery of cocoa by-products as part of a zero-waste strategy.

Keywords

Cocoa bean shells; biomass valorisation; ultrasound; hydrodynamic cavitation; methylxanthines; polyphenols.

1. Introduction

Minimising waste is one of the principles of the circular economy, which aims to transform products that are at the end of their production chain into resources for other purposes and thus close loops in industrial ecosystems (Stahel, 2016). This dissemination of sustainability awareness means that ever more attention is being paid to the reduction of waste and pollution in the agro-food industry. The

EU Waste Framework Directive (EU (2008) Directive 2008/98/EC) on food waste gives priority to waste reduction at the source, followed by recycling and reuse, leaving elimination as the last option. The majority of industries worldwide are therefore now involved in the development of environmentally friendly protocols that reduce waste production.

Food processing activities can generate by-products that contain bioactive substances with important nutritional value, benefits for human health and a market value (Helkar, Sahoo, & Patil, 2016; Martins, & Ferreira, 2017; Sharma et al., 2016). Rather than unnecessarily disposing of waste, new products can be obtained from its recycling at the end of an industrial process (Sherwood, Clark, Farmer, Herrero-Davila, & Moity, 2017). The conversion of biomass into bio-based products, which include chemicals with high added value that can be turned to beneficial use, therefore becomes an attractive business opportunity (Lai et al., 2017; Varzakas , Zakynthinos, & Verpoort, 2016).

Shells, peels, husks and skins are part of the residual materials that are being considered for agro-food industry recycling, leading to the subsequent recovery of valuable primary and secondary metabolites as well as dietary fibres to be used as supplements for food products (Galanakis, 2012). Considering the market volume of cocoa (*Theobroma cacao* L.), it is clear that substantial quantities of waste have been generated by the cocoa/chocolate industry. In 2013, 3,455,622 metric tons were produced and this has been estimated to grow at a Compound Annual Growth Rate (CAGR) of 3.1% from 2014 to 2019. The chocolate market is projected to grow further at a CAGR of 2.3% in the same period. Europe was projected to lead the global market with the highest share in terms of volume in 2014, followed by North America and Asia-Pacific (The World Cocoa Foundation, 2018). It has been calculated that 10 tons of shells are generated from each ton of dry beans produced, which creates a significant disposal problem (Vriesmanna, de Mello Castanho Ambonib, & de Oliveira Petkowicza, 2011). Cocoa shells, also known as hulls or husks, are the outer portions of beans that encase the nibs, and are a waste product of the roasting process during chocolate production. This by-product is rich in fibres and antioxidants and is a potential source of food and beverage ingredients (Martínez et al.,

2012; Vojvodić, Komes, Vovk, Belščak- Cvitanović, & Bušić, 2016). At present, this biomass is usually burnt for fuel at cocoa processing factories or used as mulch to provide nutrients for the soil and to suppress weeds. Suitable technologies to recover value-added compounds can therefore become an important economic advantage. However, this goal is still far from being fully achieved despite the release of a number of patents and papers that deal with the exploitation of by-products as a means to obtaining zero waste in the chocolate industry. Efficient biomass pretreatment, which possesses low operating costs and scalability, could facilitate the recovery of bioactive compounds from raw material. Sequential blending/sieving treatments lead to a lignin-rich coarse fraction being partially separated and nutrients being released from the matrix.

Non-conventional techniques and integrated protocols are well suited to providing a sustainable approach to biomass extraction processes. Conventional methods, such as maceration, percolation, Soxhlet extraction and hydrodistillation, are still the most common methods to obtain natural products, despite often being laborious, time-consuming, requiring large amounts of solvent and potentially causing phytochemical degradation and the partial loss of volatile compounds.

The potential for sonochemical reactors to perform green processing and provide the economic benefits of process intensification has already been established (Gogate, & Patil, 2016). Ultrasound-assisted extraction (UAE) and hydrodynamic cavitation (HC), in particular, are non-conventional and green techniques that are suitable for biomass valorisation. During UAE, low-frequency, high-intensity ultrasound (US) enhances mass transfer and the extraction process, as the physical effects of acoustic cavitation are able to prevent the thermal degradation of the matrix. The resulting cell wall disruption can dissolve or disperse compounds in a solvent, generally water, and can lead to higher component concentrations, thus reducing the need for additional processing steps (Chemat, Abert-Vian, & Cravotto, 2012; Vilkhu, Mawson, Simons, & Bates, 2008). This environmentally friendly technique is very attractive for industry thanks to its low energy consumption, safety aspects, efficiency and the possibility of easy scale-up.

HC reactors have recently seen successful use in plant extraction (Lee, & Han, 2015). The extraction process is achieved by forcing the matrix through rotating cylinders; rotor channels are periodically aligned with stator channels during high-speed rotation. Cavitation is generated when the processed liquid is accelerated in the radial direction in the cavitation chamber and subjected to a pressure wave that flows through the free channels. Cavitational bubble collapse leads to shockwaves that dramatically increase solid/liquid surface contact. Wider cavitation areas mean lower equipment costs and easier scalability, which make this reactor type more suitable for industrial applications (Rinaldi et al., 2017).

The present work aims to valorise cocoa shells by means of green protocols in order to promote the effective extraction of high-added value compounds. The potential exploitation of the waste material has been evaluated by comparing composition and physico-chemical properties, with cocoa beans as a benchmark. We have made use of our experience to optimise UAE parameters (power, extraction times) and solvents (ethanol, hexane and ternary water/ethanol/hexane mixtures) to enhance the extraction yields. The physical pretreatment of shell samples provided a fraction that was enriched in polyphenols, sugars, proteins and cocoa butter. Moreover, the extraction process has been transposed to a HC pilot reactor for pre-industrial scale-up. The efficiency of the green protocols has been evaluated by compound characterization and quantification, together with the determination of total phenolic content (Folin-Ciocalteau assay), and antioxidant activity (DPPH analysis).

2. Materials and methods

2.1 Samples

Cocoa bean shells from Ecuador were kindly provided by Gobino S.r.l. (Turin, Italy).

2.2 Chemicals

Hexane, acetone and ethanol (ACS grade, ≥99 %) (Sigma-Aldrich, Milan, Italy) were used in extractions. Methanol CHROMASOLV® (gradient grade, for HPLC, ≥99.9 %) and acetonitrile CHROMASOLV® (gradient grade, for HPLC, ≥99.9 %) for HPLC analysis were purchased from Sigma-Aldrich, while Milli-Q H_2O was obtained in the laboratory using a Milli-Q Reference A + System (Merck Millipore). Glacial acetic acid (≥ 96%) and DMSO were purchased from Merck (Milan, Italy). Standards of methyl α-D-glucopyranoside, caffeine, theobromine, methyl heptadecanoate (Me C17), gallic acid, procyanidin B2, Trolox®, the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•), the Folin-Ciocalteu reagent, sodium carbonate, *n*-butanol, formic, sulphuric, hydrochloric acids and the kit for the AOAC enzymatic method were all purchased from Sigma Aldrich.

2.3 Physical separation and extraction

The cocoa bean shells showed heterogeneous granulometry. The matrix was milled in a professional blender (Waring Blender, HGBTWTS360) at room temperature and material was subjected to physical separation using a professional sieve (Giuliani) with a mash of 1000 µm, giving two fractions according to their granulometry (Fig. 1). The finest part (FP), which was mostly deprived of the resilient fraction content (lignin and fibres), was considered for the successive protocols, whilst the coarse particles (CP) underwent ash and fibre determination analyses. Moreover, the unseparated milled matrix (TP) was considered for comparison with FP.

All the extractions (conventional, UAE and HC samples) of raw unmilled material, FP and TP have been performed with 1:10 solid/solvent ratio. Obtained samples were filtered on a paper filter placed in a Buchner funnel, and solvent phases were either separated in a separating funnel, in the case of biphasic systems (liquid/liquid), or directly evaporated to dryness to give w/w percentage extraction yields. The resulting hydro-alcoholic phase was considered a hydrophilic fraction for analytical purposes. Similarly, the lipophilic fraction was obtained from the hexane phase.

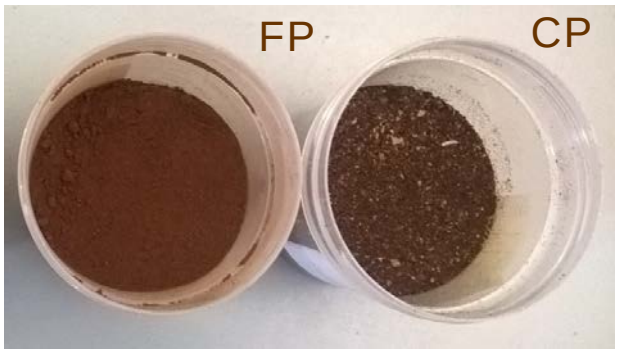

Fig. 1. The two fractions fine particles (FP) and coarse particles (CP) from physical separation.

2.3.1 US-assisted extraction (UAE)

UAE was performed using a probe system equipped with a titanium US horn (Danacamerini sas, Turin) for 15 min (150 W, 19.9 kHz) at around 40 °C. In the initial investigation, the raw material was treated with three different extraction media: hexane, hydro-alcoholic solution (70:30 EtOH/H_2O), and a ternary mixture (30:49:21 Hex/EtOH/H_2O). The optimised ternary system was also applied to TP and FP samples, in order to define the best starting material physical characteristics.

2.3.2 Conventional extraction methods

Conventional procedures were carried out with the double purpose of technique correlation and exhaustive extraction yield definition.

The temperature and extraction time conditions that were used for the UAE were then transposed for raw material treatment in a conventional magnetic stirred system. Hexane and hydro-alcoholic solvents were used. Although the ternary mixture was not ideally suited for use with magnetic stirring, the medium's efficiency was compared with those of the UAE and conventional extraction systems. The maximum yields, which correlated with matrix depletion, were defined as w/w % stressing the extraction conditions of raw material and FP fraction, and two sequential steps under reflux were performed (total 4 h).

2.3.3 Scale-up using hydrodynamic cavitation in a rotor/stator reactor

A rotor/stator cavitational reactor–that can process up to 25 L was used for scale up tests. The operating parameters at full-load were 3000 rpm for 11 min at room temperature, cycle number 47.1, cycle time 5 sec, residence time 5 sec, total residence time 3.93 min, absorbed energy 6.82 kW. Extractions were performed using the hydro-alcoholic and ternary mixtures. For the sake of comparison, the best performing ternary mixture was also used on the TP and FP fractions.

2.4 Biomass characterisation analysis

All analyses were performed in triplicate at least (three different analyses on different batch samples). Measured values are shown as an average with a confidence interval at a significance level of 0.05. All results, excluding ash content, are expressed on a dry-weight and ash-free basis.

2.4.1 Analysis of dietary fibres in physically pre-treated cocoa shells

The fibre content of the two physically pre-treated shell fractions (FP and CP) was extracted enzymatically using the AOAC method, from fat-extracted samples, and Soxlet's method (Prosky et al., 1985).

2.4.2 Ash and CHNS content

The C, H, N, S contents in cocoa beans and shells (raw material) were measured according to the EN 15104:2011 standard using a Vario MACRO elemental analyser (ELEMENTAR Analysensysteme). Ash content in the samples was measured as a residue after thermogravimetric analysis (20-650 °C, heating rate: 10 °C/min) in an oxygen atmosphere. Obtained data were in good correlation with ash content results measured by ignition at 550 ± 10 °C in a Carbolite ELF 11/6B furnace, according to the EN 14775:2010 standard (Solid biofuels - Determination of ash content).

2.4.3 Carbohydrate composition

The carbohydrate composition of the cocoa beans and shells (raw material) was determined using an alditol acetate procedure (Blakeney, Harris, Henry & Stone, 1983) after cocoa sample hydrolysis with 72% sulphuric acid. The alditol acetates were quantified by GC-FID (Agilent 6850 Series GC system) using a DB1701 column (60 m x 0.25 mm, film thickness 0.25 μm), and methyl α-D-glucopyranoside as the internal standard. Results were expressed as mannose (Man), galactose (Gal), glucose (Glc), rhamnose (Rha), arabinose (Ara) and xylose (Xyl) contents.

2.4.4 FTIR analysis

The FTIR spectra of the cocoa beans and shells (raw material) samples were recorded in KBr pellets on a Spectrum One FTIR spectrometer (PerkinElmer) in the 4000-450 cm^{-1} range (resolution 4 cm^{-1}, number of scans 64). The resulting spectra were normalised to the highest absorption intensity in each spectrum (in the ca. 3400 cm^{-1} range).

2.4.5 Pyrolyser(Py)-GC/MS/FID analysis

The Py-GC/MS/FID analyses of cocoa beans and shells (raw material) were performed using a Frontier Lab Micro Double-shot Pyrolyser Py-3030D (pyrolysis temperature 500 °C, heating rate 600 °C/s) that was directly coupled to a Shimadzu 2D FID/MS gas chromatography system MS-GC/GC-MS-2010 with a RTX-1701 capillary column (Restek, 60 m x 0.25 mm x 0.25 μm film). The injector temperature was 250 °C, the ion source 250 °C (EI 70 eV), the MS scan range m/z was 15 to 350, the carrier gas was helium (flow rate 1 mL min^{-1}) and the split ratio was 1:30. The amount of sample analysed was 1.00÷2.00 mg. The oven temperature was kept at 60 °C for 1 min, increased at 6°C/min to 270°C and finally held at 270°C for 10 min. The identification of the individual compounds was performed using GC/MS chromatograms from the Library MS NIST 14, whereas the relative peak area of individual compounds was calculated using Shimadzu software on the basis of GC/FID data. The summed molar areas of the relevant peaks were normalised to 100% and the data for 5 repetitive

pyrolysis experiments, at least, were averaged. Relative peak areas, calculated as percentages, for the pyrolysis products of differing origin were used to assess biomass sample composition. The measurement error did not exceed 5% of the mean area value.

2.5 Antioxidant activity

2.5.1 Total phenolic content (TPC)

TPC in cocoa beans and shells (raw material) was determined via a Folin–Ciocalteu analysis of the hydrophilic extracts obtained from the sequential extraction with hexane (for lipophilic removal), 70% v/v aqueous acetone and then 80% v/v aqueous ethanol (Singleton, Orthofer & Lamuela-Raventós, 1999). The use of two polar solvents leads to the complete extraction of hydrophilic extracts from cocoa biomass. 1 mL of hydrophilic extractive solution (in 50% ethanol, 150 µg/mL) was added to 0.5 mL of the Folin–Ciocalteu reagent and then gently shaken. 1 mL of 20% (w/v) sodium carbonate was added after 5 min. The solution was immediately diluted to 5 mL with distilled water and mixed thoroughly. After 10 min, the optical density of the resulting blue complex was measured at 765 nm (OD765), using a PerkinElmer Lambda 650 UV/VIS spectrophotometer, against the blank and with gallic acid, used as a standard. TPC are expressed as gram of gallic acid equivalents (GAE) per 100 g of dried sample (GAE/100 g). In order to evaluate the efficiency of cavitation in the extraction protocol, total phenolic amount was also quantified in Folin–Ciocalteu analyses in the raw, TP, FP and CP hydrophilic phases that derived from ternary mixture extracts.

2.5.2 Oligomeric proanthocyanidin content (OPC)

The OPC in the hydrophilic extracts of cocoa beans and shells (raw material), obtained according to the procedure described above, were quantified using the butanol-HCl method with procyanidin B2 as the reference compound (Schofield, Mbugua, & Pell, 2001).

2.5.3 Determination of antioxidant activity using the DPPH• radical scavenging method

The radical scavenging ability of all the hydrophilic phases from ternary mixture extracts was evaluated using the stable free radical DPPH•, according to the method described by Brand-Williams, Cuvelier & Berset (1995). Details of the procedure and calculations are reported by Boffa *et al.* (2016). All samples were prepared in triplicate, and DPPH• radical scavenging activity was expressed as µg dry extract per mL solution ± standard deviation. Trolox® equivalents (TE) µmol/g of extract were calculated according to EC50 values.

2.6 Instrumental analyses

2.6.1 Methylxanthine determination by HPLC-DAD

Caffeine and theobromine contents were determined for the hydro-alcoholic phases of the raw, TP, FP and CP samples that were extracted with the ternary mixture under US irradiation and for the raw material under optimized HC treatment. HPLC analyses were performed on a Waters binary pump 1525 linked to a 2998 PDA (Waters Corp., Milford, USA), using a Synergi Hydro RP C18 column (250 mm, 4.6 mm, 5 µm; Phenomenex, Torrance, California, USA) and 2% acetic acid (A) and acetonitrile (B) as mobile phases. The monitored wavelength was 280 (PDA range 200-600 nm). The gradient program started from 0% B, which was maintained for 6.5 min, up to 50% B over the 6.5-30 min period, from 50% to 100% B over 30-36 min, followed by a 100% B step over 36-42 min. Caffeine and theobromine standard solutions (from 0.02 to 2 mg/mL) were analysed by HPLC (20 µL injection) to give linear regressions with R^2 >0.999. Before the injection (20.0 µL), all samples were dissolved in MeOH, giving concentrations of between 10 and 20 mg/mL.

2.6.2 GC analysis of fatty acid methyl esters (FAMEs)

The fatty acid composition of the lipophilic (hexane) phase, from the ternary mixture extracts of the raw, TP, FP and CP samples, was determined according to the procedure described by Bermúdez Menéndez *et al.* in 2014. GC-MS qualitative analyses were performed in an Agilent Technologies

6850 Network GC System, using a 5973 Network Mass Selective Detector, a 7683B Automatic Sampler (Santa Clara, California, USA), and a capillary column (HP-5MS 5% Phenyl Methyl Siloxane, length 30 m, i.d. 0.25 mm, film thickness 0.25 μm). GC-FID quantitative analyses were performed in an Agilent Technologies 7820A Network GC System equipped with a FID detector, using a capillary column (Mega WAX, length 30 m, i.d. 0.25 mm, film thickness 0.25 μm, Mega S.r.l., Legnano, MI, Italy), according to the internal standard amount (methyl heptadecanoate, Me C17). All the lipophilic extracts (~10 mg) were derivatised before analysis (see Grillo et al. 2018: Bermúdez Menéndez et al. in 2014 detailing GC operating conditions and sample derivatisation). FAME identification was performed by checking how samples corresponded with C8–C24 saturated and unsaturated external standards (Sigma-Aldrich), which were prepared in solution with GC grade cyclohexane, and with Wiley7n and NIST11 GC libraries (for GC-MS analysis).

2.6.3 UHPLC-ESI-MS/MS analysis of polyphenols

3 mg of the hydrophilic fraction, which was obtained from the extraction of the FP sample with the ternary mixture under US irradiation, were dissolved in a 1:1 acetonitrile/water mixture, filtered over a nylon filter (0.45 μm pore size) and analysed by UHPLC-ESI-MS/MS. An Acquity UPLC system (Waters Corp., Singapore) that was coupled with a quadrupole-time of flight (Q-TOF) MS instrument (UPLC/Synapt Q-TOF MS, Waters, Milford, MA, USA) and had an electrospray ionisation (ESI) source was used. A U-HPLC column (2.1 mm x 50 mm i.d., 1.7 μm, BEHC18, Waters Acquity) was used at a flow rate of 0.30 $mL \cdot min^{-1}$. The mobile phases were water with 0.1% formic acid (A) and acetonitrile (B). The gradient program was: 0-0.5 min, 5% - 5 % (B); 0.5 - 10 min, 5% - 95 % (B); 10 - 15 min, 95% - 95 % (B). The injection volume was 2 μL. The major operating parameters for Q-TOF MS were set as follows: capillary voltage, 2 kV (-); cone voltage, 40 V; cone gas flow, 50 L/h; collision energy, 4 eV; source temperature, 120 °C; desolvation temperature, 350 °C; collision gas,

argon; desolvation gas, nitrogen; flow rate, 600 L/h; data acquisition range, m/z 50–1.200 Da; ionisation mode, negative.

3. Results and discussion

3.1 Cocoa beans and shell characterisation

A recent review reported that cocoa shell composition varied considerably by origin and processing protocol (Okiyama, Navarro & Rodrigues, 2017). As an indicative average value, shell fibre content is almost three times that of the nibs, and constitutes over 50% of the entire material (Martín-Cabrejas, Valiente, Esteban, & Mollà, 1994). Furthermore, fat amount is slightly lower than in the nibs and presents higher variability (between 20 and 185 g/kg of the dried material). Finally, the quantity of proteins is similar in the two matrices, but only about 1% of it exists in the free condition.

The approximate compositions of the cocoa shells and a cocoa bean sample were defined and compared in this work, using FTIR (Bellamy, 1980; Faix, 1992), CHNS analysis, analytical pyrolysis (Py-GC/MS/FID) and wet chemical analytical methods. These analytical results demonstrate the high residual value of this waste matrix, validating and consolidating the concept of valorisation.

The FTIR spectrum of the cocoa shells showed lower absorption intensity in the aliphatic and protein regions (range 2918-2851 cm^{-1}, amide I and II bands at 1630 and 1549 cm^{-1}, respectively), which indicates that shells have much lower aliphatic compound (presumably lipids) and protein content than the beans (see Table 1 and Fig. 1 in Grillo et al., 2018). This observation was confirmed by the decreased H/C atomic and N/C atomic ratio observed in the elemental analysis data shown in Table 1 (see Fig. 2 in Grillo et al., 2018).

Table 1. Elemental composition of whole cocoa beans and shells, expressed as w/w percentage on oven dried ash free biomass.

Sample	Moisture	Ash[a]	Elemental content [a]			
	w/w %	w/w %	C	H	N	O
Cocoa shells	6.66	9.0±0.1	50.3±0.1	5.7±0.03	3.5±0.01	40.6±0.1
Cocoa beans	3.68	6.1±0.05	52.6±0.1	6.3±0.05	4.6±0.01	36.6±0.1

[a] w/w % on oven dried biomass

Moreover, cocoa shells showed significantly lower absorption intensity at 1152 cm^{-1} (C-O-C asymmetric vibration in carbohydrates and glucosides) and 1518 cm^{-1} (aromatic skeletal vibrations) than cocoa beans, which may indicate that they have lower cellulose and phenolic contents (see Fig. 1 in Grillo et al., 2018). Data on sugar content from the analytical pyrolysis of cocoa samples confirmed that shells contain a lower quantity of glucose, but more rhamnose and galactose (possibly originating from pectin substances), as well as mannose and xylose (possible components of non-cellulosic cell wall polysaccharides) than cocoa beans (see Table 2 in Grillo et al., 2018).

Polysaccharides and alkaloids were found to be the major components of the cocoa materials under study (see Table 3 in Grillo et al., 2018, main summarized in Table 2). The fact that cocoa shell samples are characterised by higher alkaloid content, mainly theobromine, than cocoa beans can be explained by the migration of theobromine during the bean fermentation stage, as described in the above-mentioned review. A comparison of the portions of aliphatic-derived volatiles (including amides and nitriles of fatty acids) in analytical pyrolysis products revealed that the highest lipid and fatty acid contents were found in the beans (16.8 + 3.6% rel.) and the lowest (4.6 + 1.9% rel.) in the Ecuador cocoa shells. The relative phenolic compound contents in the volatile analytic pyrolysis products were very similar for all cocoa samples investigated (about 8% rel.).

Table 2. Summarised results of the analytical pyrolysis GC/MS/FID analysis of cocoa samples including GC diagnostic peak assignments and relative contents (w/w %) of carbohydrates, lipids, lignin and other polyphenol-, alkaloid- and protein-derived products detected in volatiles.

Volatiles (compound/group of compounds)	**Precursors**	**w/w %**	
		Beans	**Shells**
Acids, esters, aldehydes, ketones, cyclopentane and furan derivatives, sugars	Carbohydrates	37.21	44.28
Phenyl and benzyl derivatives	Lignin + Polyphenols	7.70	7.76
Aliphatic compounds	Lipids + Fatty acids	16.83	4.63
Alkaloid-derived volatiles	Alkaloids	34.15	38.75
3,7-dihydro-1,3,7-trimethyl-*1H*-purine-2,6-dione	Caffeine	3.44	4.53
3,7-dihydro-3,7-dimethyl-*1H*-purine-2,6-dione	Theobromine	20.76	25.30
Amides and nitriles	Lipids + Proteins	3.57	1.87

The cocoa shell samples differed from the beans in that they had higher relative contents of lignin specific diagnostic compounds, namely guaiacol, 4-vinyl guaiacol, syringol and 2,3-dihydro-benzofuran, in the pyrolysis products. Phenol and *p*-cresol dominated the phenolic analytical pyrolysis products for all cocoa samples under study, while catechol was detected in the volatiles in low (but not negligible) quantities. These results suggest that the polyphenolic pool of cocoa samples under study contains tannins, together with some amounts of lignin, thus confirming the literature data. The cocoa shell samples showed the lowest content of volatiles from protein-pyrolysis-derived (portions of amides and nitriles).

Literature data on the total polyphenolic contents of cocoa products reported values in the 0.3-6.5% range for the powder and 1.3-1.8% for the shells (Arlorio *et al.*, 2005; Arlorio, Coisson, Restani, &

Martelli, 2001; Lecumberri *et al.*, 2007). The yields of phenolic compounds (see Paragraph 2.5.1) that could be extracted from the cocoa samples under study were rather high (9.2 and 4.7% for cocoa beans and shells, respectively) although they are close to the range reported in the literature. The enhanced values of detected polyphenols may be connected to the use of two-step extraction with different polar solvents, while the larger amounts obtained in the pyrolysis data come from the depolymerisation of lignin. The results of the wet-chemistry (butanol-HCl) analysis of the hydrophilic extract confirmed that oligomeric proanthocyanidins (polymerisation degree ≥2) are the main constituents of the soluble polyphenolic fraction of the cocoa samples under study (Fig. 2).

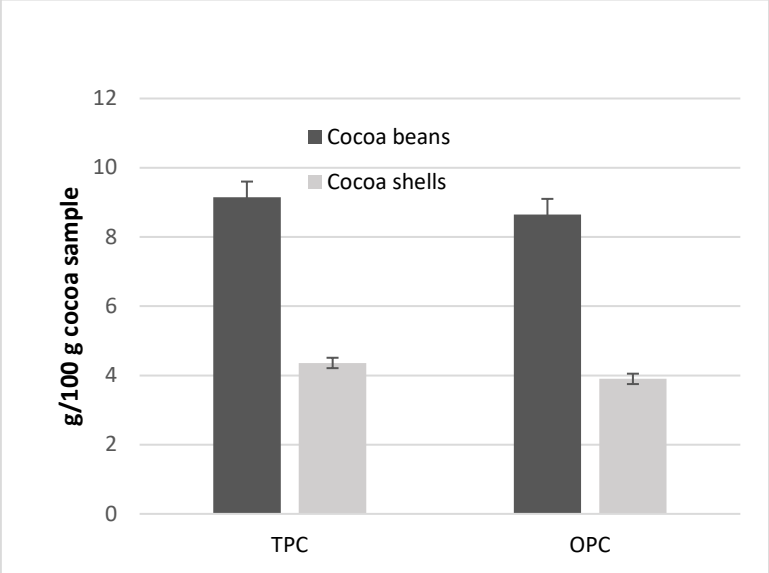

Fig. 2. Total phenolic content (TPC) and oligomeric proanthocyanidin content (OPC) in the cocoa samples, expressed as GAE/100 g and w/w %, respectively, of the oven-dry ash-free sample.

3.2 Raw cocoa shell extraction

To date, few papers have studied the extraction of fibres, pectins, polyphenols, fats and theobromine from cocoa shells as a means to produce low-calorie, fibre-rich substances and antioxidant additives to be included in foods, dietary supplements, pharmaceuticals and cosmetics (Okynama et al., 2017).

The first part of this work was focused on a general screening of solvent systems and cavitation technologies in order to define an optimized extraction protocol for cocoa shell material in the raw form, as obtained by the production plant.

3.2.1 Solvent Screening - UAE vs conventional extraction

The first step was to discover the best solvent system with which to recover fats and obtain a polyphenol-enriched fraction from cocoa shells in synergy with US cavitation, developing a rapid and efficient extraction process. Pure hexane and a 70:30 EtOH/H_2O mixture were chosen to fractionate the lipophilic and hydrophilic compounds (Amin & Yee, 2006). A second protocol, using a 30:49:21 Hex/EtOH/H_2O ternary mixture was performed to test the one-step extraction of molecules of different polarity. A 15 min extraction time was chosen to optimise both extraction yields and energy savings for these preliminary experiments. The data reported in Table 3 show that the ternary solution increased extraction yields in a single, fast and efficient treatment of the matrix. Moreover, the use of the 30:49:21 Hex/EtOH/H_2O mixture allowed both the hexane and hydro-alcoholic phases to be easily recovered after filtration.

For the sake of comparison, UAE parameters, in terms of time, temperature and S/L ratio, were reproduced in silent conditions. As Table 3 shows, US always increased both lipophilic and hydrophilic extracts yields. It is worth noting that the use of the ternary mixture in a conventional system reduces the lipophilic extract yield, probably because of the poor mass-transfer. The effectiveness of UAE was evaluated using quantitative data that was obtained when carrying out an exhaustive reflux extraction under magnetic stirring, using the hexane and hydro-alcoholic solutions separately. This comparison demonstrates that the best results under sonication were reached using the ternary mixture, which gives slightly lower total hydrophilic fraction recovery and provides a 16-fold reduction in extraction time.

Table 3. Ultrasound assisted extraction (UAE) and conventional process yields from the raw cocoa shells, expressed as w/w percentage.

Solvent	**UAE**	**Conventional**	
		Silent	**Reflux**
Hex	1.5	0.6	11.0
70:30 EtOH/H_2O	9.6	6.9	15.0
30:49:21 Hex/EtOH/H_2O	2.5/13.9[a]	1.3/8.2[a]	-

[a] Extraction yields of hexane and hydro-alcoholic phases, respectively.

3.2.2 US and HC - Technology comparison and scale up

The encouraging results obtained with UAE and the ternary solvent mixture can be enhanced further using a new source of cavitation. HC is able to simultaneously grant intimate mixing between solvent and shells and easy scale-up.

A screening was performed to demonstrate the feasibility of extraction scale-up while increasing yield. The optimised ternary mixture was transposed into a pilot scale protocol under HC; the raw matrix was processed in a rotor/stator reactor (Rotocav®) (Crudo et al., 2016), which allowed up to 25 L of solvent and 2.5 kg of matrix to be used. For the sake of comparison between HC and UAE, extractions were also carried out with the 70:30 EtOH/H_2O mixture, and thus confirmed the greater efficiency of the first process (extraction yield of 14.8% *vs* 9.6%). The ternary mixture afforded a slight increase in the hydrophilic extract under HC (14.6% *vs* 13.9%), and much higher percentage of lipids (10.1% *vs* 2.5%) than UAE. The data in Table 4 also show that the efficiency of acoustic cavitation in the presence of EtOH was maximised when used in the ternary mixture (13.9% *vs* 9.6%), while HC was not affected by this factor (14.6% *vs* 14.8%). Pure hexane was not considered for use

with the HC system, due to its physical properties (mainly low evaporation point and high flammability).

Table 4. Ultrasound assisted extraction (UAE), hydrodynamic cavitation (HC)-assisted extraction and conventional quantification yields from the raw cocoa shells, expressed as w/w percentage.

Solvent	**UAE**	**HC**	**Reflux**
Hex	1.5	- [a]	11.0
70:30 EtOH/H_2O	9.6	14.8	15.0
30:49:21 Hex/EtOH/H_2O	2.5/13.9[b]	10.1/14.6[b]	-[a]

[a] Not performed. [b] Extraction yields of hexane and hydro-alcoholic phases, respectively.

An evaluation of the technologies, using extraction yields, confirms the effectiveness of the ternary solution, which actually improved on UAE results when coupled with the rotor-stator reactor. Additional analysis and deeper characterization of the extracts were performed to support process validation. Antioxidant activity and methylxantine content (namely caffeine and theobromine) were determined for the hydrophilic fraction, whilst the lipophilic extract underwent FAME quali-quantification.

Table 5. Theobromine and caffeine content, total phenolic content (TPC), antioxidant activity and fatty acid methyl esters (FAMEs) content, respectively, for the hydro-alcoholic and hexane phases obtained from the extraction of raw cocoa shells with the 30:49:21 Hex/EtOH/H_2O mixture in different conditions.

Tech.	**Hydro-alcoholic phase**	**Hexane phase**

	Theobromine		Caffeine		TPC		DPPH EC50	Trolox eq	FAMEs	
	w/w % extract	mg/g shells	w/w % extract	mg/g shells	mg/g extract	mg/g shells	µg/ml	µmol/g extract	w/w % extract	mg/g shells
US	5.04	7.02 ± 0.11	0.81	1.13 ± 0.09	51.1	7.1	76.9 ± 3.6	204.7 ± 9.6	91.7	23.2
HC	9.25	13.5 ± 0.16	0.75	1.09 ± 0.12	79.9	11.7	72.1 ± 4.1	218.3 ± 12.4	94.3	95.5

The theobromine and caffeine content of the hydro-alcoholic phases obtained from the extraction of raw cocoa shells with the 30:49:21 Hex/EtOH/H_2O mixture was determined using HPLC-DAD analysis and calibration curves of both alkaloid standards (Table 5). While the caffeine amount does not seem to be particularly influenced by the extraction method used, hydrodynamic cavitation in the Rotocav® gave the highest theobromine yield values, almost twice as much as the nearest contender. TPC, $DPPH^{\bullet}$ radical scavenging activity (EC50, expressed as µg dry extract per mL solution ± standard deviation) and Trolox® equivalent (TE) (µmol/g of extract, according to EC50) values were determined to measure the antioxidant power of the extracts. According to results reported in Table 5, the TPC gap between the two examined samples was not reflected by a significant spread of the other parameters. This fluctuation may be related to the differing reactivities of the analytical methods, whose responses can change because of a variety of interfering molecules. The FAME content of the hexane phases obtained from the extraction of raw cocoa shells using the 30:49:21 Hex/EtOH/H_2O mixture was calculated after a transesterification/esterification derivatisation process, where triglycerides and free fatty acids were transformed into methyl esters. Quantitative data were calculated using GC-FID chromatograms, based on Me C17 internal standard amounts. As can be seen in Table 5, the detected FAME percentages in the hexane phase were very high for both the extracts. HC was the most efficient, recovering almost 4-times as many fats from raw biomass (95.5 *vs* 23.2 mg/g shells) than UAE. Qualitative determination was performed using GC-MS analysis, and comparing mass spectra with databases (NIST11 or Wiley7n MS libraries). The

identification of almost all esterified compounds (98.7%) permit us to state that the average composition of FAMEs (see Table 5 in Grillo et al., 2018) is almost identical in all the samples analysed. In particular, the detected profile appears to match the cocoa butter benchmark, in which palmitic, stearic and oleic acids are the most abundant (Lipp & Adam, 1998).

3.3 Matrix effect

The role of the matrix has been evaluated starting from the optimized protocols on raw cocoa shells, achieved with US and HC. A physical pre-treatment/separation step was considered in order to define larger application possibilities and better understand shell valorisation. The removal of fibre-rich components, which are not valuable from the extraction point of view, from the starting biomass allowed a "noble fraction" to be isolated. This procedure leads to increased yields as the extraction is focused on an enriched matrix and thus avoids recalcitrant material that will burden separation/purification steps and increase the final disposal mass. The pre-treatment can also enhance mass-transfer, increasing particle surface area, which is a desirable side effect. Besides these considerations, it is crucial to state that the "poor fraction", which results from physical separation, does not represent a material loss or residue generation. Conversely, it can be exploited as a fibre source for fodder, flour or for mulching. The waste/product ratio of the cycle can be reduced and thus optimise energy consumtion at the same time.

3.3.1 Fraction Analyses – Enrichment validation

Raw cocoa bean shells were roughly milled in a professional blender, while room temperature was maintained, and then divided into two quantified fractions, according to their granulometry. FP is the finest part and mostly lacks the resilient components (lignin and fibres) contained in CP. The effectiveness of the separation was investigated using a dietary fibre and ash content comparison.

Table 6. Ash and fibre content of physically pre-treated shell fractions, determined respectively by thermogravimetric analysis and AOAC protocol, expressed in w/w percentage on dry material.

Fraction	w/w % dry shells	Ash	Dietary Fibres		Tot Fibres
			Soluble	Insoluble	
FP	30	2.7	13.01	41.44	54.45
CF	70	6.3	15.84	65.52	81.36

In the reported comparison between the two fractions in Table 6, a decrease of c.a. 67% in total fibre content can be seen for FP, along with half the ash concentration. Backed by these results, the FP was considered for successive extraction investigations, together with the unseparated milled matrix (TP), used for comparison. TP has the role of defining the influence of fibre removal and excluding the mere effect of comminution.

3.3.2 Extraction screening

The fractions obtained (TP, FP) were subjected to UAE and HC under the optimised conditions with the 30:49:21 Hex/EtOH/H_2O mixture. Extraction yields were considered a first, general investigation to define process trends to be used in parallel with conventional quantifications, which were obtained in separated extractions with hexane and the hydro-alcoholic mixture.

Table 7. Ultrasound assisted extraction (UAE) and hydrodynamic cavitation (HC)-assisted extraction yields with the ternary solution from total milled particles (TP) and fine particles (FP) fractions in comparison with conventional quantitative yields, obtained from separated extractions with hexane and the hydro-alcoholic mixture (expressed as w/w percentages).

Matrix	Process	Hydro-alcoholic phase	Hexane phase

TP	UAE	13.8	2.5
	HC	14.7	10.3
	Reflux[a]	14.9	11.3
FP	UAE	19.4	3.7
	HC	20.5	15.8
	Reflux[a]	20.9	16.1

[a] Separated extraction steps with hexane and hydro-alcoholic mixture.

The results confirm that the effects of fractionation are not simply reducible to matrix comminution (Table 7). In particular, FP gave higher yields for lipophilic and hydrophilic extracts than both TP and the raw samples (see Table 4), which is confirmed by the exhaust quantification. Data from TP indicate that rough grinding did not affect total extraction yields. The HC protocol provided the best results for all the matrixes, nearly depleted the biomass in every application and led to the maximum results when used coupled with the FP fraction (20.5% and 15.8% for hydrophilic and lipophilic fractions, respectively).

The above-mentioned extraction data were complemented by the same characterization method as above (Paragraph. 3.2.2.), the results of both will be discussed in parallel later in the text.

A comparison of the characterisation data from the raw material and TP fraction extracts (Table 5 and 8), uncovers common behaviour and shows that rough milling had little impact on extract properties. According to this outcome, HC tests were not performed on this cocoa matrix.

Methylxanthine analyses gave the top results for the FP fraction with a general increase in caffeine and theobromine content, with the latter showing the highest growth. An evaluation of the ratio between w/w % and mg/g shells showed that the effectiveness enhancement, over the others, seen in

FP extraction could be explained by higher extract mass, both for US and HC. The scale up system gave the best performance and achieved superior methylxantine concentration (16.02 and 0.89 w/w % of theobromine and caffeine on FP extract, respectively). Considering the mean percentage values of theobromine (1.3%) and caffeine (0.2%) reported for cocoa, processed raw cocoa shells contain these methylxanthines in comparable amounts (Table 5). The theobromine extraction yields increased when using the FP fraction, giving a value near 3% (32.7 mg/g shells, Table 8). The huge amounts of theobromine and caffeine that were found in the pyrolysis analysis were probably not available for extraction under our conditions.

Data from the Folin-Ciocalteau evaluation were almost comparable with those reported in the literature (Amin & Yee, 2006; Arlorio *et al.*, 2001; Lecumberri *et al.*, 2007). Obtained TPC values generally ranged from 50 to 125 mg/g extract (Table 5 and 8), close to the 113 mg/g extract obtained by Amin using an ethanolic solvent, whilst the HC-assisted protocol on FP shows a peak result of 197.36 mg/g extract. The best US extract (fraction FP, ternary mixture) gave 24.3 mg/g shells, slightly higher than the value obtained by Arlorio (18 g/kg shells), but lower than Lecumberri's results (58 g/kg shells). This gap was greatly reduced when HC was coupled with the enriched fraction FP, increasing yield up to 40.44 mg/g shells. Antioxidant activity trends reflect the phenolic concentration of the samples, highlighting the superiority of FP with both US and Rotocav®, reaching 62.03 ± 3.1 µg/ml for EC50 and 256.69 ± 9.9 Trolox eq µmol/g shells.

Table 8. Theobromine and caffeine content, total phenolic compounds (TPC), antioxidant activity and fatty acid methyl esters (FAMEs), respectively, for hydro-alcoholic and hexane phases, obtained from the extraction of total milled particles (TP) and fine particles (FP) fractions with the 30:49:21 Hex/EtOH/H_2O mixture in different conditions.

Matrix	**Tech.**	**Hydro-alcoholic phase**								**Hexane phase**	
		Theobromine		**Caffeine**		**TPC**[a]		**DPPH EC50**	**Trolox eq**	**FAMEs**	
		w/w % extract	mg/g shells	w/w % extract	mg/g shells	mg/g extract	mg/g shells	µg/ml	µmol/g extract	w/w % extract	mg/g shells
TP	US	3.47	4.8 ± 0.10	0.72	1.00 ± 0.08	51.9	7.18	83.1 ± 5.3	189.4 ± 12.1	91.5	23.1
	HC[b]	-	-	-	-	-	-	-	-	-	-
FP	US	9.14	17.8 ± 0.09	0.73	1.43 ± 0.07	125.0	24.3	66.9 ± 2.4	235.3 ± 8.4	93.7	34.4
	HC	16.02	32.70 ± 0.12	0.89	1.76 ± 0.08	197.4	40.4	62.0 ± 3,1	256.7 ± 9,9	96.4	141.6

[a] Expressed as gallic acid equivalents. [b] Not analysed.

The hexane phases obtained from the extraction of FP and TP with the 30:49:21 Hex/EtOH/H_2O mixture in different conditions were characterized using FAME evaluation, confirming the process intensification shown by HC over UAE. The FP fraction (Table 8) showed a significant increase in mg/g shells values (around 50%) over the raw cocoa shells (Table 5) (34.4 *vs.* 23.2 mg/g shells for UAE, 141.6 *vs.* 95.5 for HC).

According to all previous analyses and considerations, the optimized extraction protocol can be defined as a combination of a milled fine fraction of cocoa shells, with the coarse residues removed, a 30:49:21 Hex/EtOH/H_2O solution and a HC scale-up reactor. After 11 minutes of processing, which corresponds to c.a. 4 minutes of total residence time (inside the rotating chamber), the solution gave, in high yields, the richest extract, in terms of TPC and antioxidant activity, caffeine and theobromine concentration, together with a valuable lipophilic fraction.

In order to better investigate phenolic composition, UHPLC-ESI-MS/MS analyses were performed on the best hydrophilic extract (FP fraction under HC with the ternary mixture) and were compared with the two conventional extracts (obtained with 70 % v/v aqueous acetone and 80 % v/v aqueous EtOH) that were used for total extractable polyphenol determination (see TPC determination). The total ion chromatogram is shown in Fig. 3, with related peak assignation in Table 9. For every compound, corresponding mass fragments and, when available, literature references for assignation are reported.

Information on polyphenolic composition in cocoa shells is scarce in the literature. In cocoa beans, however, the main polyphenols are reported to be flavan-3-ols (epicatechin, catechin and proanthocyanidins) with small amounts of anthocyanins and flavonols (quercetin glycosides) also being present (Vriesmann, de Mello Castanho Ambonib, & de Oliveira Petkowicza, 2011).

Conventional extracts (see Fig. 3 A and B, Table 4 in Grillo et al., 2018) showed the following compounds: catechin, epicatechin, procyanidin dimers, trimers and tetramers, as well as a number of tetramers that may be proanthocyanidin derivatives. Furthermore, bioactive flavonoids (quercetin and its derivatives, mainly glucosides), non-flavonoid polyphenols (hydroxycinnamic acid derivatives) and carboxylic acids (citric acid) were found. The FP fraction HC-assisted extract obtained using the ternary mixture (only hydro-alcoholic phase, Fig. 3) showed the partial depolymerisation of procyanidins, apparently caused by cavitation, as the intensities of compounds 4 and 6 decreased and peak 15 was not detected, while lipophilic compound intensities (11 and 12) in the UHPLC-MS spectra slightly increased.

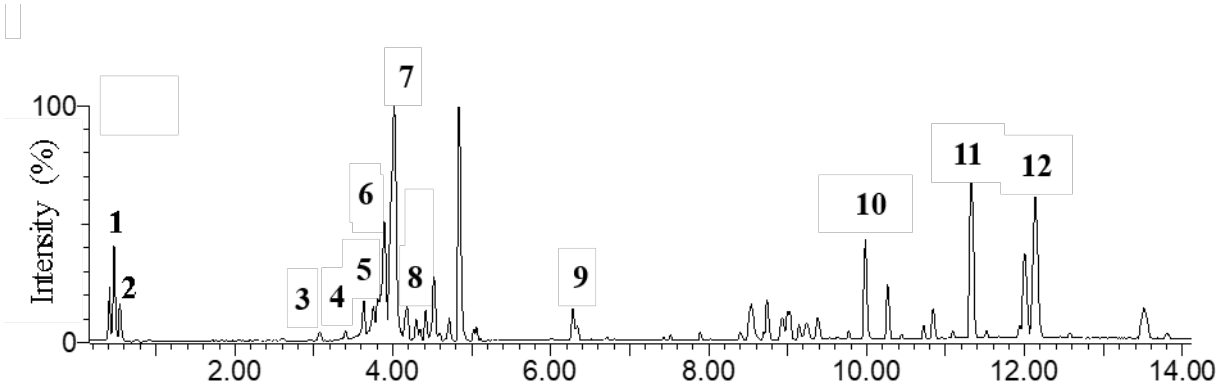

Fig. 3. Total ion chromatogram (negative ionisation) resulting from the UHPLC-ESI-MS/MS analysis of the hydrodynamic cavitation (HC) extracts of fine particles (FP) fractions using the ternary mixture, obtained from Ecuador cocoa shells.

Table 9. Polyphenols detected in ESI^- TIC, resulting from the UHPLC-ESI-MS/MS analysis of the extracts obtained from cocoa shells (Fig. 3).

Compound	**Peak Nr.**	**[M-H], Main fragments**	**Ref.**
Gluconic acid sodium salt/glucose acid	1	195, 177, 129, 85, 75	Karim, 2014
Citric acid	2	191, 111, 87	Karim, 2014
Protocatechuic acid	3	153, 109, 65	Karim, 2014
Procyanidin tetramer	4	1153, 577, 289	Hammerstone, 1999
N-caffeoyl-*L*-aspartate derivative	5	276, 179, 131	Pereira-Caro, 2013
Procyanidin trimer	6	865, 860, 577, 305, 289, 245	Hammerstone, 1999
Catechin or epicatechin with a cinnamic acid side-group	7	633, 329, 305, 289, 267, 225	Reed, 2009
Catechin/epicatechin derivative	8	289, 245,205,179	-
Flavone/luteolin	9	329, 311, 229, 211, 171, 139, 127	Karim, 2014
Hydroxybenzoic acid sugar derivative	10	299, 137	-
Linoleic acid	11	279	Karim, 2014
Oleic acid	12	281	-

3 Conclusions

This work has focused on the core of the valorisation process, while maintaining an awareness of sustainability principles. It has worked not only on the lab-scale, but also paves the way for effective industrial application. A fast procedure that can feasibly and efficiently be up-scaled has been designed. Furthermore, the impact of possible pre-treatment steps has been evaluated. The final extracts, both hydrophilic and lipophilic, have been thoroughly characterized, while their high added value and the fact that they approach matrix depletion (98% of extraction efficiency, if compared to conventional quantification) have been highlighted.

This cascade protocol, which includes physical separation followed by green extraction, demonstrates that cocoa shells are a valuable source of antioxidant flavanols (catechin and epicatechin), methylxanthines (theobromine, 160.2 mg/g extract and caffeine, 8.9 mg/g extract), fatty acids (964 mg/g extract) and fibres. Lab-scale results have been confirmed and enhanced in a pilot reactor, giving yields of around 15.8% w/w in cocoa butter and around 20.5% w/w in hydrophilic compounds, composed mainly of polyphenols and methylxanthines, for pre-treated shells. The high TPC of the hydro-alcoholic fraction (197.4 mg/g extract) is reflected into a significant radical scavenging and antioxidant activity (62.0±3.1 μg/mL in DPPH EC_{50} and 256.7±9.9 μmol/g extract in Trolox eq.).

Finally, the fibre-rich fraction, removed via physical treatment, can be used as feed or mulch, while the exhaust lignocellulosic material from extraction can be further converted without purification into sugars or fine chemicals using a microwave-assisted depolymerisation processes (Carnaroglio et al. 2015; Tabasso, Montoneri, Carnaroglio, Caporaso, & Cravotto, 2014;), which would close the valorisation cycle.

With the aim of developing a zero-waste cacao industry in mind, a new cocoa shell valorisation process has been designed that is benign by construction and attempts to follow a circular economy

approach. Exploiting a vegetal residual biomass fulfils several sustainability criteria; using a renewable feedstock and preventing the accumulation of waste at the same time, while also providing process “input-pushing, via the use of an abundant and cost-effective matrix. The cavitational reactor used in this work can generate high-energy microenvironments, as well as significantly promote biomass deconstruction in short times and with low energy consumption. The key role played by enabling technologies, such as US and HC, is an easy way to satisfy the significant and simultaneous demands of minimizing energy and maximizing efficiency. Furthermore, merging defatting pre-treatment (necessary to increase total yields) with the extraction step, allows the whole process to be halved in size, focusing energy use and completely avoiding dedicated work-up. Moving from a multistage to a single-step process means unifying work-ups, removing dedicated equipment and solvents, and providing energy savings. In addition, producing extracts from a food source grants traceability and an absence of contaminants in the matrix. This prerequisite in essential for a suitable nutraceutical product to provide active compounds without synthetic steps. These results should facilitate the valorisation of cocoa shells and help create a zero-waste strategy for the cocoa industry.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2018.08.057.

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